Hedgehog (Hh) signaling controls gene transcription through the Gli family of transcription factors, including Drosophila Ci. In the absence of Hh, Ci acts as a transcriptional repressor, but upon signaling activation, Ci becomes a transcriptional activator. Two Hh target genes, patched (ptc) and decapentaplegic (dpp) contain enhancers with Ci binding sites. The ptc enhancer has three high-affinity Ci sites, whereas the dpp enhancer has only low-affinity sites. In the developing wing, ptc is expressed in a narrow stripe of cells that receive the highest levels of Hh, while dpp is expressed in a broader stripe in a region of moderate signaling. We found that dpp requires low-affinity sites for optimal activation by Hh, as replacing the low-affinity binding sites of dpp with high-affinity sites from ptc caused repression of dpp in cells that receive moderate signaling. Because higher binding affinity correlates with increased Ci occupancy of these enhancers, our results are consistent with the idea that Ci may act more cooperatively as a repressor than as an activator in cells that have both forms of this transcription factor. To test this hypothesis, we quantified the expression of synthetic enhancers with three high-affinity sites versus one high-affinity site. Our data showed that having a single high-affinity site abolishes the transcriptional repression observed in enhancers with multiple high-affinity sites. These results are consistent with the cooperative repression model, thus we propose a novel transcriptional mechanism to interpret the Hh signaling gradient.

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Program/Abstract # 556
The mutational basis for the repeated evolution of a cis-regulatory element generating morphological diversity
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A central goal of evolutionary developmental biology is to elucidate the gradual progression of mutational steps by which development, and thereby traits evolve. Empirical and theoretical studies implicate mutations in cis-regulatory element (CRE) sequences, which control gene expression, as a prominent route by which development evolves. However, few studies have determined both the mutational (the identity of the evolutionarily relevant mutations) and molecular (biochemical property altered) basis of CRE evolution. Hence, this type of evolutionary path remains poorly understood. One excellent model trait to study CRE evolution is the diverse abdominal pigmentation patterns exhibited by species of the Drosophilinae subfamily. These patterns have evolved by modifications to a well-characterized gene regulatory network. Male-specific sexually dimorphic pigmentation of *Drosophila melanogaster* is a particularly tractable trait controlled by the Bric-à-brac (Bab) transcription factor proteins. Previously, we identified a CRE controlling sexually dimorphic Bab expression, and elucidated how it functions and evolved in one lineage. Here we show that alterations in this CRE contribute to pigmentation variation within a species and furthermore differences in orthologous dimorphic elements similarly correlates to pigmentation differences between closely-related species. Using ancestral reconstruction methods, we determined the sequence and gene regulatory activity of the dimorphic element possessed by various ancestors at key phylogenetic nodes. Moreover, here we present data that has begun to trace the mutational and molecular mechanistic path by which descendant CREs with distinct activities evolved.

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Program/Abstract # 557
Premature differentiation and reversal of imprinted X-Chromosome inactivation in extraembryonic ectoderm lacking paternally derived Xist
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Through the process of X-Chromosome Inactivation (XCI), somatic cells of mammalian females inactivate one of their two X-Chromosomes in order to balance X-linked gene dosage with their male counterparts. During mouse embryogenesis, two forms of XCI are observed, imprinted and random. Imprinted XCI inactivates the paternally inherited X-Chromosome (Xp) and occurs in the extraembryonic lineages. Random XCI occurs in the embryonic lineages where either the Xp or the maternally inherited X-Chromosome (Xm) can be inactivated. The process of XCI is dependent upon the long non-coding RNA Xist, which is expressed from and coats the inactivated X-Chromosome (Xi) in cis. Consequently, females harboring a paternally derived Xist mutation (Xist+/−) die due to failure of imprinted XCI and poor trophoblast development. Here, we investigate the consequence of two active X-Chromosomes in the extraembryonic ectoderm (ExE) of Xist+/− female embryos. At embryonic day 6.5, we find that the Xist+/− ExE no longer proliferates and lacks the transcriptional regulator Cdx2, a factor required to maintain the ExE in a progenitor state. Curiously, we observe an Xi in a few cells of the ExE. When grown in culture, Xist+/− embryo outgrowths retain Cdx2 and harbor an Xi in some Cdx2+ cells. Trophectodermal stem cells derived from Xist+/− embryos harbor an inactive Xm, consistent with a reversal of imprinted XCI. Taken together, our data suggests that poor trophectoderm development in Xist+/− embryos is due to premature differentiation of the trophoblast progenitors. Furthermore, the capability of Xist+/− ExE cells to reverse imprinted XCI suggests that the ExE no longer retains the initial imprint required for imprinted XCI.

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Program/Abstract # 558
A role for Xenopus Zygote Arrest 2 (Xzar2) in the regulation of key cell cycle mRNAs
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Zygote Arrest proteins, Zar1 and Zar2 (aka Zar1-like) have been implicated in the oocyte to embryo transition, zygotic genome activation, preimplantation development and epidermalization, and disruption of these proteins causes zygotic arrest at the 1 or 2-cell stage in mouse embryos. However, the mechanism of action of Zar proteins is unknown, transcriptional regulation, chromatin remodeling and RNA metabolism, have all been suggested. Early development is regulated by maternal mRNAs that have specific combinations of cis-elements in their 3′ untranslated regions (UTR) that determine where, when and to what extent each mRNA is translated. The mRNA of a key cell cycle regulator of embryogenesis, Wee1, is translated during oocyte maturation. The Wee1 mRNA 3′ UTR contains a cis-element called the Translation Control Sequence (TCS) that regulates mRNA translation during meiotic maturation of Xenopus oocytes. The protein that binds to the TCS is unknown. Here, we show that